ALGAL TOXINS AND APEX PREDATORS: DOMOIC ACID IN THE THRESHER SHARK, *Alopias vulpinus*, IN THE SOUTHERN CALIFORNIA BIGHT

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Introduction

Thresher sharks are highly valued in the fresh fish trade and are the leading commercial shark in California, with a habitat range from Clarion Island, Mexico to British Columbia, Canada (Holts et al. 1998; Compagno 2001).

In the California Current, thresher shark diet consists of small schooling fish such as northern anchovy (*Engraulis mordax*), Pacific sardine (*Sardinops sagax*), Pacific mackerel (*Scomber japonicus*), Pacific hake (*Merluccius productus*) and invertebrates such as market squid (*Loligo opalescens*) (Preti et al. 2001; 2004).

Coastal pelagic species (northern anchovy, Pacific sardine, and market squid) are known to be potent vectors for domoic acid (DA), a neuroexcitatory toxin produced by several species of the diatom genus *Pseudo-nitzschia*, because they can consume large quantities of the toxic diatoms and remain unimpaired (Lefebvre et al. 2012; Bargu et al. 2008).

Some of the major events involving the fatal ingestion of DA include the following:

1991: an abnormal number of deaths were reported for Brandt's cormorants (*Phalacrocorax penicillatus*) and brown pelicans (*Pelecanus occidentalis*) on the US West Coast, caused by the ingestion of DA-containing northern anchovies, a toxic vector (Work et al. 1993; Lefebvre et al. 1999).

1996: Pacific mackerel consumption caused a massive die-off of 150 brown pelicans in Baja California, Mexico (Beltran et al. 1997).

1998: over 400 California sea lion (*Zalophus californianus*) deaths were recorded, also caused by the ingestion of DA-containing northern anchovies (Scholin et al. 2000; Lefebvre et al. 1999).

The combination of location and diet makes the thresher shark a prime target for exposure to DA.

Determining levels of DA in thresher sharks will help reveal current exposure risk and help elucidate potential future changes.

Methods and Results

Sampling

Stomach and blood samples were collected during the NMFS Juvenile Thresher Shark Abundance Survey conducted aboard commercial fishing vessel *Outer Banks* from September 9–26, 2011 off the Southern California Coast from Ventura south to San Diego. Samples were collected within 5 miles of shore in depths ranging from 12 to 20 m.

Sharks were caught on longlines baited with mackerel or sardine, brought on board, and data was collected on length, sex, location of capture, as well as hydrodynamic variables at the catch location such as sea-surface water temperature, color, and depth. Blood was drawn from the caudal vein, or directly from the heart when animals suffered a mortality, using an 18g, 1.5" needle.

Stomach contents were collected from sharks that suffered mortality by excising the entire stomach, and securing the pyloric and esophageal ends. Stomachs and blood were frozen and kept frozen until analysis.

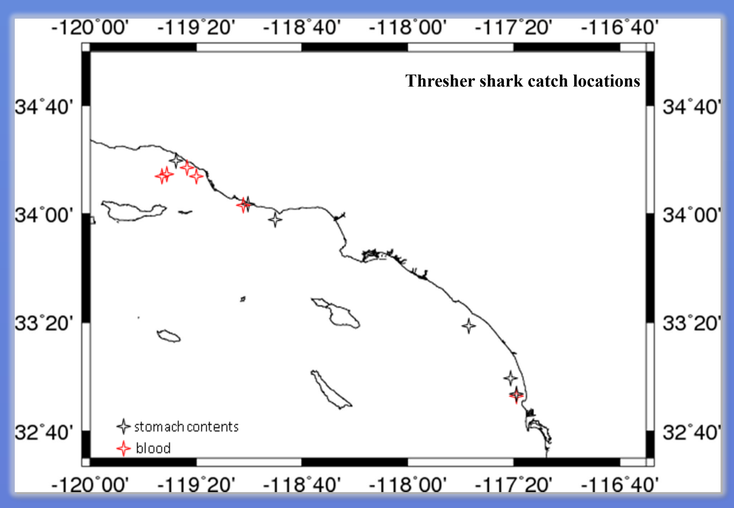

Figure 1. Collection locations of thresher shark stomach contents (black crosses) and blood (red crosses).

Stomach content analysis

Stomach contents were enumerated, weighed, and identified following Preti et al. 2001. Prey items with tissue attached were considered for the DA analysis. A portion of about 10 g of muscle tissue for fish and mantle for cephalopods was dissected, put in vials and plastic bags and refrozen for later analysis.

Extraction and quantification of DA

Samples analyzed for DA were extracted in 50% aqueous methanol at a ratio of 1:4 (sample:methanol). Extracts were homogenized then centrifuged; the supernatant was filtered and samples were stored at 4°C until analysis. DA was quantified using Biosense® ELISA (Biosense® Laboratories, Bergen, Norway) according to the manufacturer's instructions with modifications made to the minimum extract dilutions in order to avoid matrix effects. For more detailed methods description see Lefebvre et al. 2010.

Methods and Results, Cont.

A total of eight prey items from six shark stomachs and six blood samples were analyzed for DA. Stomach contents and blood samples were taken from different specimens, except in one case where both stomach contents and blood were taken from the same shark.

The prey items with the highest DA values were market squid (44.6 ng/g) and Pacific sardine (39.1 ng/g). Three blood samples were positive for DA with the highest having a concentration of 47.9 ng/g. This value is one of the highest recorded in blood from a field-collected animal.

Shark ID	Date	Sample	DA ng/g
TS1	9/11/2011	prey item (Pacific sardine)	39.1
TS2	9/15/2011	prey item (market squid)	7.6
TS3	9/19/2011	prey item (market squid)	44.6
TS3	9/19/2011	prey item (Pacific mackerel)	10.3
TS4	9/21/2011	prey item (Pacific sardine)	10.5
TS5	9/22/2011	prey item (Pacific mackerel)	4.5
TS5	9/22/2011	prey item (northern anchovy)	*bdl*
TS6	9/23/2011	prey item (northern anchovy)	*bdl*
TS6	9/23/2011	blood	0.9
TS7	9/10/2011	blood (heart blood)	*bdl*
TS8	9/10/2011	blood	13.6
TS9	9/11/2011	blood	*bdl*
TS10	9/14/2011	blood	*bdl*
TS11	9/15/2011	blood	47.9
bdl = below reliable limit of detection (stomach contents: 2.0 ng/g, blood: 0.4 ng/g)			

Table 1. Domoic acid levels in stomach and blood samples from thresher sharks

UPLC-tandem mass spectrometry confirmation of DA in thresher shark blood

Ultra high performance tandem mass spectrometry was used to confirm the presence of DA. UPLC-MS/MS is a technique used to indentify compounds by determining the mass of the unknown compound and that of characteristic ion fragments created when the molecule is broken apart.

System: Waters UPLC system coupled to a triple quadrupole tandem mass spectrometer (MicroMass, Waters) run in positive ion mode using multiple reaction monitoring (MRM)

Column: Acquity BEH C18 micro-particulate column (1.7 µm, 50 mm x 2.1 mm, Waters)

Flow rate: 0.8 ml/min Temperature: 40°C Injection vol. 20 µl

Mobile phase: (A) water (B) 95% acetonitrile, both with 50m formic acid

Separation: linear gradient from 5-15% B for 3 min, followed by a 30 sec. hold at 15% B, then a 90 sec re-equilibration to 5% B.

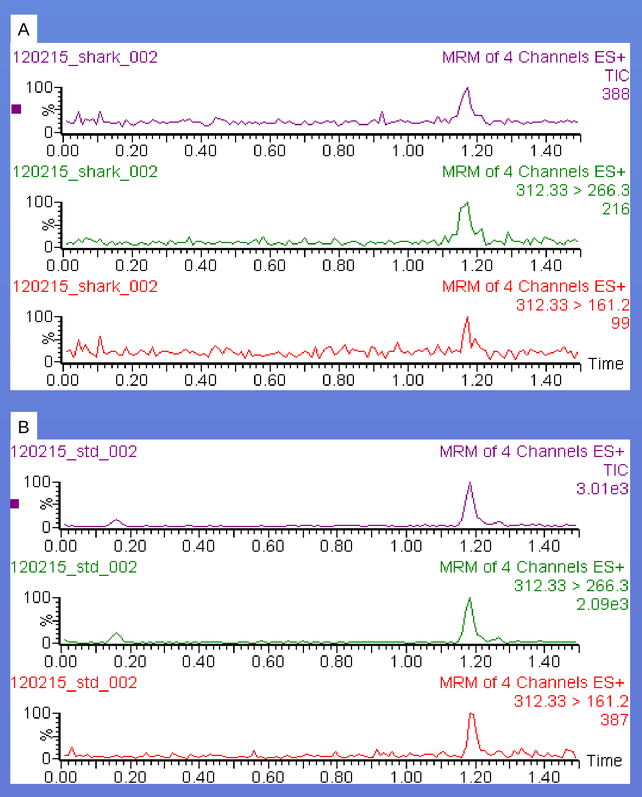

Figure 2. UPLC-MS/MS traces from (**A**) thresher shark blood (sample TS11) and (**B**) DA standard. The purple trace shows the total of all ion fragments, while the green and red traces represent the two most abundant transition ions.

Conclusions

- **Thresher sharks are exposed to DA through their prey.**
- **Despite our small sample size (n=6) DA was found in 50% of the blood samples collected. In previous studies California sea lions and other marine mammals had a significantly lower percentage of DA hits in blood samples.**
- **The shark specimen (TS11) with the highest blood DA concentration was caught by the tail, indicating that the toxin did not impair its ability to hunt and stun prey with the tail.**
- **The study samples do not contain a concentration of DA that would cause symptoms or death; however, if a large bloom of toxic *Pseudo-nitzschia* occurred, sharks could be exposed to higher doses of DA which might cause symptoms.**
- **It is also possible that even at higher DA doses thresher sharks may not display symptoms if they share the ability of other fish (such as anchovy and sardine) which are able to consume large quantities of DA without apparent ill-effects.**
- **The work of Schaffer et al. 2006 speculates that leopard sharks might have some unknown mechanism for dealing with DA. They show that leopard sharks possess the molecular target for DA but are resistant to injected doses of DA known to be toxic to other vertebrates.**
- **Due to small sample size and lack of previous studies on DA toxicity in sharks, more research is needed.**

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